## REMARKS

### Status of the Claims

Claims 1-19 and 21 are pending. Claims 20 and 22 were previously cancelled by Applicant without prejudice or disclaimer. Claims 5, 7-14 and 16-18 were withdrawn from consideration by the Examiner. Claims 1-4, 6, 15 and 19 and 21 are under consideration.

### Amendment of Claims

Claims 1, 3 and 15 were amended to replace "intracellular" with "intracellularly." Claims 1, 3, 4, 6, 15, 19 and 21 were amended to add the word "step." Claims 4 and 6 were amended to delete the word "above." All these amendments are intended to improve the language of the claims and they do not add new matter. Claims 1, 3 and 15 were amended to replace "subpopulation of" with "integrin alpha 10-chain expressing" and to remove "wherein said subpopulation has enhanced chondrogenic potential as compared to the rest of the mammalian mesenchymal stem cell population." These amendments find support throughout the specification. See, for example, Patent Application Publication No. 2005/0221327 A1, at Abstract and ¶¶ 48-50, 158-161, and 227-239. Accordingly, none of the amendments adds new matter.

### Rejection under 35 U.S.C. § 112, first paragraph

**Claims 1-4, 6, 15, 19 and 21** were rejected under 35 U.S.C. § 112, first paragraph, as allegedly "containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Office Action, page 2. Specifically, the Office contends that the phrases "a

subpopulation of mammalian mesenchymal stem cells" and "wherein said subpopulation has enhanced chondrogenic potential as compared to the rest of the mammalian mesenchymal stem cell population" introduced limitations into the claims which were not clearly disclosed in the specification and recited in the original claims.

Applicant respectfully submits that this rejection is moot because Applicant has amended the claims to remove these phrases, as described above. In light of this, Applicant respectfully requests that the rejection of claims 1-4, 6, 15, 19 and 21 under 35 U.S.C. § 112, first paragraph, as lacking written description be withdrawn.

**Claims 1-4, 6, 15, 19 and 21** were also rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Office Action, page 3. The Office maintained this rejection for the same reasons set forth in the previous Office Action mailed on May 23, 2008 ("Office Action I"). In Office Action I, the Office stated that the factors most relevant to this rejection are the scope of the claims, the amount of direction or guidance provided, the lack of sufficient working examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention. Office Action I, page 3. Applicant respectfully traverses this rejection for the reasons outlined in the Reply to Office Action mailed September 19, 2008, and the following additional reasons.

(i) Not all MSCs have to express integrin alpha 10

The Office stated as one reason for the rejection that "the hMSCs must express the marker recognized by the 'molecule' in order for the method to work. The specification fails to demonstrate that all MSCs express the claimed marker." Office Action I, page 3 (emphasis added). The Office further argued that "Applicant has

identified a subpopulation of hMSCs that express integrin alpha 10 chain." Office Action I, page 4 (emphasis added). Applicant respectfully submits that this reasoning does not apply to the currently pending, amended claims, because the amended claims relate to the use of a marker for, or to the identification of, integrin alpha 10 chain-expressing MSCs. Hence, whether all MSCs or only a subpopulation of MSCs express integrin alpha 10 is irrelevant with respect to the enablement of the amended claims.

The Office cited Varas et al. (2007) and Lundgren-Akerlund et al. (2006) in support of its argument that Applicant has identified a subpopulation of hMSCs that express integrin alpha 10 chain. Office Action I, page 4. Applicant points out that these references support Applicant's amended claims. The references confirm that human MSCs express integrin alpha 10 chain ("We also found that human MSCs express integrin α10," Lundgren-Akerlund et al.; "Our results suggest that α10 can be used as a marker for highly proliferative MSCs with good chondrogenic differentiation potential," Varas et al., page 976).

(ii) Integrin alpha 10 can be used as a marker for MSCs

The Office also argued that it is unclear how one skilled in the art can use α10 as a marker for MSCs because Varas et al. did not detect expression of integrin alpha 10 chain by FACS in bone marrow aspirates directly after preparation, i.e., at day 0, or by immunohistochemical analysis of whole bone sections. Office Action I, pages 4, 6 and 7. Applicant points out that Varas et al. explain this difficulty detecting alpha 10-positive cells because of the low frequency of MSCs in bone marrow ("Considering the reported low frequency of MSCs in BM ($1:10^5$ to $1:10^6$), it is unlikely that we would detect α10-positive cells if α10 is a true marker of MSCs in BM," Varas et al., page 976, 1st col.,

2nd ¶).

The fact that the frequency of MSCs in bone marrow is low does not mean, however, that integrin alpha 10 chain can not be used as a marker for MSCs. First, integrin alpha 10 chain can be used to identify MSCs in other tissues. For example, Varas et al. were able to detect integrin alpha 10 expression by immunohistochemistry in the endosteum and the periosteum, both of which are tissues known to contain mesenchymal progenitor cells. See Varas et al., page 971, 1st col., 1st ¶.

Second, integrin alpha 10 can be used to identify MSCs from bone marrow after expansion of these cells. For example, Example 3 of the specification provides a procedure for using an integrin alpha 10 chain as a marker for MSCs derived from bone marrow, involving the expansion of MSCs from a fresh bone marrow preparation by growth of adherent cells in culture. Applicant submits that one of ordinary skill in the art at the time the application was filed would have had no difficulty using the procedure provided in Example 3. Furthermore, the Office already indicated that the specification is enabling for a method for identifying integrin alpha 10 chain expressing MSCs if the procedure includes FGF-2 treatment of a sample, as described in Example 3.

(iii) The claimed methods do not require FGF-2 treatment

The Office also appears to argue that the specification is only enabling for a method for identifying integrin alpha 10 chain-expressing MSCs if the method comprises the step of "contacting the sample with FGF-2." Office Action, page 3.

In this respect, the Office stated that "the specification, while being enabling for a method for identifying an integrin alpha 10 chain expressing mesenchymal stem cells comprising a) providing a sample comprising mesenchymal stem cells, b) contacting the

sample with FGF-2, c) contacting the sample in step b) with an antibody which specifically binds integrin alpha 10 chain, d) detecting integrin alpha 10 expression on the cell surface of cells of the sample or intracellular in cells of the sample, e) positively correlating the integrin chain alpha 10 expression detected in step e) with the cells being the alpha 10 expressing mesenchymal stem cells, does not reasonably provide enablement for the claimed methods recited in 1-4, 6, 15, 19 and 21." Office Action, page 3. The Office also stated that Example 3 of the specification only supports FGF-2 treated MSCs. Office Action, page 2.

Applicant respectfully disagrees that the specification is only enabling for a method that includes a FGF-2 treatment step, for the following reasons. The experiment disclosed in Example 3 analyzed the expression of integrin alpha 10 on bone marrow-derived cells that were cultured with or without FGF-2 for two weeks prior to analysis by FACS using a monoclonal antibody against alpha 10. Figure 4 shows results for the FGF-2 treated cells but not for the non-treated cells. The Office appears to require step b) ("contacting the sample with FGF-2") for enablement because Figure 4 only shows test results for the FGF-2 treated cells.

However, FGF-2 treatment is not required for the claimed methods to work, and nothing in the specification suggests that it is. The objective of Example 3 was to test whether colony-forming cells derived from human bone marrow express the integrin alpha10 and represent a population of mesenchymal stem cells. See specification, ¶¶ 228-229 in US 2005/0221327 A1 ("[0228] Objective [0229] To test whether colony-forming cells derived from human bone marrow express the integrin alpha10 and represent a population of mesenchymal stem cells."). The experimental approach

included cell cultures that were grown with or without FGF-2. It was known at the time of filing that FGF-2 can promote the growth of MSCs (see specification, ¶ 184 in US 2005/0221327 A1), but it was also known to one skilled in the art that FGF-2 treatment is not essential for growth of MSCs (see Declaration by Evy Lundgren-Akerlund, dated June 9, 2009, page 3). Thus, not surprisingly, both types of cell cultures, i.e. those grown with or without FGF-2, contained a significant percentage of integrin alpha 10 chain-expressing MSCs, with a higher percentage in the FGF-2 treated cultures (see Declaration by Evy Lundgren-Akerlund, dated June 9, 2009, pages 3-4). Qualitatively, both types of cell cultures yielded similar results, namely that colony-forming cells derived from human bone marrow express the integrin alpha 10 chain and represent a population of mesenchymal stem cells. Only the percentage of such integrin alpha 10 chain-expressing MSCs was higher with FGF-2 treatment (see Declaration by Evy Lundgren-Akerlund, dated June 9, 2009, pages 3-4). The results from both types of cell cultures described in Example 3, i.e. those grown with or without FGF-2, provided the same answer, namely that colony-forming cells derived from human bone marrow express the integrin alpha10 and represent a population of mesenchymal stem cells. Thus, it is Applicant's opinion that at the time of filing the application, showing the results of either type of cell culture, i.e. grown with or without FGF-2, was sufficient to provide the answer to the question being addressed according to the stated objective. Nothing in Example 3, or the rest of the specification, suggests that the cell cultures grown without FGF-2 would yield a qualitatively different result with respect to the stated objective of Example 3.

The results obtained for the FGF-2 treated and non-treated cells have since been

confirmed, for example, in Varas et al. (cited by the Examiner in Office Action I; see, e.g., Figure 6; see also Declaration by Evy Lundgren-Akerlund, dated June 9, 2009, page 4).

Applicant therefore respectfully submits that treatment of a sample comprising MSCs with FGF-2 was not necessary to enable one skilled in the art to use the claimed methods. The percentage of integrin alpha 10-expressing MSCs in a sample may be lower if grown without FGF-2, but that does not affect the ability of one skilled in the art to use the claimed methods.

Furthermore, Applicant notes that nothing in the application suggested to one skilled in the art that the claimed methods can only be used with a FGF-2 treatment step. Thus, one of ordinary skill in the art would have had no reason to believe that the claimed methods could not be used without a FGF-2 treatment step. And, importantly, one of ordinary skill in the art would have been successful in using the methods without a FGF-2 treatment step, as was Applicant when conducting the experiments described in Example 3, and as was Varas et al. and others. No undue experimentation was required. One of ordinary skill in the art simply had to follow the directions and guidance provided in the specification, including in Example 3.

In summary, Applicant submits that the full scope of the amended claims, which now specifically relate to integrin alpha 10-expressing MSCs, is enabled. Applicant also submits that the specification, including the working examples, provide sufficient direction and guidance for one of skill in the art to use the claimed methods. Finally, Applicant submits that the directions provided, for example, in Example 3, are detailed enough to enable one of ordinary skill in the art to use the claimed methods without

significant experimentation.

In light of the above remarks, Applicant submits that one of ordinary skill in the art at the time the application was filed would have been able to use the claimed methods, including methods of using integrin alpha 10 as a marker for MSCs and of identifying MSCs, without undue experimentation. Accordingly, Applicant respectfully requests that the rejection of claims 1-4, 6, 15, 19 and 21 under 35 U.S.C. § 112, first paragraph, as lacking enablement be withdrawn.

## Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims. If the Examiner believes a telephone conference would be useful in resolving any outstanding issues, the Examiner is invited to call the undersigned at (202) 408-4316.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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